SEARCHING FOR THE RELATIVES OF Coffea (Rubiaceae, Ixoroideae): THE CIRCUMSCRIPTION AND PHYLOGENY OF Coffeae Based on Plastid Sequence Data and Morphology

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The circumscription of Coffeeae (Rubiaceae) and phylogenetic relationships within the tribe were evaluated using sequence data from four plastid regions (trnL-F intron, trnL-F intergenic spacer [IGS], rpl16 intron, and accD-psal IGS) and a morphological data set. Eleven candidates for inclusion in Coffeeae were examined using plastid data, and a further three were investigated using morphology alone. Based on previous phylogenetic analysis of the subfamily Ixoroideae, nine genera representing five tribes were used as outgroups. Our results support an enlarged circumscription for Coffeeae, containing 11 genera, viz. Argocoffeopsis, Belonophora, Calycosiphonia, Coffea, Diplospora, Discopernum, Nostolachma, Psilanthus, Tricalysia, Sericanthe, and Xantonnea. The inclusion of Diplospora and Tricalysia within Coffeeae, based on published molecular data, and the inclusion of Argocoffeopsis, Belonophora, Calycosiphonia, Discopernum, and Sericanthe, based on morphological evidence, are well supported. Nostolachma is newly transferred from Gardenieae subtribe Diplosporinae to Coffeeae, and Xantonnea from Octotropideae to Coffeeae. The exclusion of Bertiera from Coffeeae and placement in tribe Bertiereae is supported on the basis of molecular and morphological data. The removal of Diplospora and all other genera from Gardenieae subtribe Diplosporinae to Coffeeae and Octotropideae renders Diplosporinae superfluous. It is proposed that Xantonneopsis be transferred to Octotropideae; Petitiocodon is tentatively placed in Gardenieae. The monophyly of seven genera is supported, but Coffea is identified as paraphyletic in relation to Psilanthus on the basis of molecular and combined molecular and morphological data.

Key words: accD-psal; Coffea; coffee; Coffeeae; molecular systematics; morphology; rpl16; Rubiaceae; trnL-F.

Before this can happen, however, it is necessary to investigate the relationship between these two genera and other closely related groups within Rubiaceae.

Coffea comprises 96 species and occurs naturally in Africa, Madagascar, and the Mascarenes (Davis et al., 2006). A close relationship between Coffea and Psilanthus Hook. f. is evident from studies covering several disciplines. Careful morphological study by Robbrecht and Puff (1986) and Robbrecht (1988b, 1994) has shown that the two genera share several key features and on this basis were considered the component genera of tribe Coffeeae DC. (Robbrecht and Puff, 1986). Couturon et al. (1998) produced a fertile intergeneric hybrid via the crossing of C. arabica and P. ebracteolatus Hiern, and genetic correspondence has been further revealed by recent cytological studies (Lombello and Pinto-Maglio, 2003, 2004). Coffea and Psilanthus have also been the focus of several recent phylogenetic studies, using systematic data from various sources, including morphology (Stoffelen, 1998; Davis et al., 2005), random amplified polymorphic DNA (RAPD) (Lashermes et al., 1993), sequences from plastid DNA (Cros, 1994; Lashermes et al., 1996; Cros et al., 1998), and ITS sequences of nuclear ribosomal DNA (Lashermes et al., 1997). At the species level, the studies of Lashermes et al. (1997) and Cros et al. (1998) have provided the most useful data: they were able to separate Coffea species into geographical groupings and to begin to examine the relationships between Coffea and Psilanthus species. Lashermes et al. (1997) found that one Psilanthus species (P. travancorensis) was nested within Coffea and that there was limited sequence divergence.

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between these two genera. They concluded that their ITS data did not support the recognition of two genera. On the basis of trnL-trnF sequence data, Cros et al. (1998) concurred with Lashermes et al. (1997) about the close relationship between Coffea and Psilanthus, although their tree topology shows an unresolved relationship between the two species of Psilanthus that they sampled (P. manii and P. ebracteolatus) and Coffea. Cros et al. (1998) and Lashermes et al. (1997) did not include representatives of closely related genera in their studies, for example, as outgroups. However, broader studies of Rubiaceae (Ixoroidae) by Andreasen et al. (1999) and Andreasen and Bremer (2000, fig. 3) infer paraphyly of Coffea.

Potential relatives of Coffea should be found within Coffeaceae, but at the present time there is no general agreement on the constituent genera of the tribe (Robbrecht and Puff, 1986; Robbrecht, 1988b, 1994; Andreasen and Bremer, 1996, 2000; Persson, 2000; Bridson and Verdcourt, 2003) and few data on how they are related to one another (Andreasen and Bremer, 2000).

The nomenclatural origin of tribe Coffeaceae is De Candolle’s Coffeaceae (De Candolle, 1807), one of four tribes recognized by De Candolle in his early classification of Rubiaceae. In a later conspectus of the family by De Candolle (1830), which includes 13 tribes, it is evident that his Coffeaceae (“Coffeaceae”) was very broadly circumscribed and included a large number of genera, many of which have since been placed in different tribes and subfamilies. De Candolle’s characterization of Coffeaceae translated and reorganized by us from the Latin diagnosis (De Candolle, 1830, p. 472), is as follows: Trees or shrubs with opposite leaves; stipules interpetiolar, paired, adnate or free. Ovaries 1-locular, each bearing a single ovule. Fruits fleshy, containing two pyrenes, each containing a single seed with a bony-crustaceous endocarp (endocarp layer of pyrene), endocarp flat on the adaxial surface and usually sulcate on the abaxial surface, or fruits with one pyrene by abortion; endosperm hard/horny.

Richard (1830) provided a similar concept for Coffeaceae, although it should be noted that De Candolle (1830) had access to Richard’s unpublished manuscript and incorporated parts of the classification in order to avoid nomenclatural conflict (Stern, 1957).

Further attempts to define Coffeaceae were not made until much later. Leroy (1980) included three genera in his informal ‘Caféiers’ group, namely Coffea, Nostolachma T. Durand, and Psilanthus, although no direct reference was made to this tribe by Leroy. Robbrecht and Puff (1986; see also Robbrecht, 1988b, 1994) provided the first rigorous and explicit delimitation for Coffeaceae and included just two genera in the tribe, Coffea and Psilanthus. Their characterization of the tribe was based on the presence of 2-carpellate ovaries, each with a single ovule, axile placentaion, a hard (homy/crustaceous) endocarp, seeds with a deep L- or T-shaped ventral groove on the adaxial surface (as seen in transverse section; endocarp and seed coat invaginated), and a seed coat exotesta consisting of thin, elongated parenchymatous cells usually containing many more or less isolated fibers (derived from Robbrecht, 1981; Robbrecht and Puff, 1986). In addition, Stoffelen et al. (1997) showed that the pollen of Coffeaceae are (2-)3–5-colporate (zonocolporate). A simple but efficient means of characterizing Coffeaceae sensu Robbrecht and Puff (1986) is of a tribe possessing “coffee beans,” i.e., seeds with a groove on the flat side of the seed. The groove ramifies within the seed as an invagination through the endosperm into the center and is clearly evident when a coffee bean is cut in transverse section. The pyrene (homy/crustaceous endocarp) also has a deep, ventral groove that follows the invagination within the seed. Robbrecht and Puff (1986) excluded Nostolachma (= Lachnostoma Korth.), which was associated with Coffea and Psilanthus by Leroy (1980). Nostolachma, together with Argocoffeopsis Lebrun, Calycosiphonia Pierre ex Robbr., Cremaspora Benth., Diplospora DC., Sericanthae Robbr., and Tricalysia A. Rich. ex DC. were transferred to Gardenieae DC. subtribe Diplosporinae Miq. by Robbrecht and Puff (1986). It was not explicitly stated by Robbrecht and Puff (1986) why and how Gardenieae subtribe Diplosporinae was separated from Coffeaceae, although it seems that the absence of characters found in Coffeaceae (as discussed earlier) coupled with the presence of exotestal cells (with or without thickenings and lacking fibers) separated these two groups. Petitiocodon Robbr. and Xantonneopsis Pit. were added to this subtribe by Robbrecht (1988b), followed by Discospermum Dalz. (Ali and Robbrecht, 1991; Robbrecht, 1994) on the basis that they conformed to the circumscription of the subtribe (after Robbrecht and Puff, 1986).

Molecular studies (Andreasen and Bremer, 1996, 2000; Persson, 2000) have demonstrated that certain genera of Gardenieae subtribe Diplosporinae are closely related to Coffea and Psilanthus. On the basis of molecular data, Andreasen and Bremer (2000) placed two genera of Gardenieae subtribe Diplosporinae, Diplospora and Tricalysia, with Coffea and Psilanthus, in Coffeaceae. The genus Paracoffea L.-F. Leroy (Leroy, 1967) used in that work is a synonym of Psilanthus (Davis, 2003). Bertiera Aubl., formerly either a genus of unknown systematic position within Rubiaceae (Robbrecht, 1988b) or placed within Gardenieae subtribe Gardeniinae (Robbrecht et al., 1994), was also shown to be closely related to Coffea and Psilanthus (Andreasen and Bremer, 1996, 2000; Persson, 2000). Andreasen and Bremer (2000) placed Bertiera in Coffeaceae, based on a moderately well-supported (BP = 76, b = 4) sister relationship to Coffea, Psilanthus, Diplospora, and Tricalysia. Andreasen and Bremer (2000) placed two further members of Gardenieae subtribe Diplosporinae, Discospermum and Sericanthe, into Coffeaceae, based on the morphological evidence (Schumann, 1891; Ali and Robbrecht, 1991) that the former was closely related to Diplospora and the latter to Tricalysia. Andreasen and Bremer (2000) placed Cremaspora, formerly included in Gardenieae subtribe Diplosporinae by Robbrecht and Puff (1986), in its own tribe (Cremasporae Bremek, ex S.P. Darwin).

An enlarged and modified concept of Coffeaceae (see Table 1) was followed by Bridson and Verdcourt (2003, p. 387) although further significant changes were made. Based on morphology (discussed later) and on preliminary plastid sequence data that we provided (A. Davis, unpublished data), Bridson and Verdcourt (2003, p. 451) added the genera Argocoffeopsis, Belonophora Hook. f., and Calycosiphonia to Coffeaceae. Argocoffeopsis and Calycosiphonia were formerly members of Gardenieae subtribe Diplosporinae (Robbrecht, 1988b), and Belonophora was tentatively included in Aulacocyaceae Robbr. & Puff (Robbrecht, 1988b, 1994). Bridson and Verdcourt (2003, p. 388) excluded Bertiera from Coffeaceae because they believed that the extension of characters necessary to accommodate this morphologically distinct genus would greatly distort the circumscription of Coffeaceae. To this end, Bertiera was placed in its own tribe, Bertieraeae (K.Schum.) Bridson (Bridson and Verdcourt 2003, p. 386).
The differences between the tribes were not stated, but instead the characterizations for each tribe were given in tribal descriptions (Bridson and Verdcourt, 2003, pp. 387–388). The salient characters of Coffeeae according to Bridson and Verdcourt (2003, p. 388) are as follows (we selected the main characters and the comments in square brackets are ours): the inflorescences are axillary and paired at nodes (in some genera accompanied by terminal inflorescences) and ovules per locule 1–few (only in some Tricalysia spp. more than 20). In subtribe Bertierinae the inflorescences are terminal and ovules per locule numerous.

Following the work of Robbrecht and Puff (1986), Andreasen and Bremer (1996, 2000), Persson (1996), Bridson and Verdcourt (2003), and Robbrecht and Manen (2006), the aims of this study are to further investigate the delimitation and characterization of Coffeeae and to elucidate the relationships within Coffeeae with particular attention to the position of Coffea and Psilanthus. To achieve our objectives, we used sequence data from four plastid regions (the trnL intron, the trnL-F intergenic spacer [IGS], the rpl16 intron, and the accD-psaI IGS) and compared and combined these data with morphological data. For the purposes of this study, the trnL intron and the trnL-F IGS are treated as one region: trnL-F. All genera with potential to be placed within Coffeeae, including all members of Gardenieae subtribe Diploropinae (based on Robbrecht, 1988b, 1994), were included in the sampling, although we were unable to locate DNA material for three genera from Gardenieae subtribe Diploropinae. Morphological data alone were used to deduce the systematic position of genera not sampled in our plastid sequence analyses.

### MATERIALS AND METHODS

**Taxon sampling**—Species representatives from all genera placed within the current enlarged concept of Coffeeae by Andreasen and Bremer (2000) and Bridson and Verdcourt (2003) were included in our analyses, viz. Argocoffeopsis, Belonophora, Bertiera, Calycosiphonia, Coffea, Diploropina, Disco...
sporophyll, Petitiocodon, Sericanthe, and Trialsytra. For the three remaining
genera of Gardenieae subtribe Diplosperinae (Robbrecht, 1998b), our
material of Nostolachma, Petitiocodon, and Xantoneoidea did not yield usable
DNA and plastid sequences could not be obtained. Petitiocodon, Xantoneoidea,
and Nostolachma were included in the morphological analysis and then in a
combined morphological–molecular analysis to determine their systematic
position. A review of genera within Rubiaeae (A. Davis and D. Bridson, Royal
Botanic Gardens, Kew, personal observations) identified one further genus
likely to be placed within Coffeaeeae namely Xantonea Pierre ex Pit. This was
included in the plastid sequence and morphological analyses. Collections made
in 2005 from Cameroon and the Congo (B. Sonké, University of Yaoundé,
personal communication) yielded a new taxon that was likely to fall within
Coffeaeeae, although as yet it cannot be placed within a currently accepted genus.
This taxon is currently referred to (B. Sonké and A. Davis, personal
communication) as Calycosiphonia cf, as it superficially resembles species
within this genus. All genera, and their respective samples, with the potential to
fall with the broader concept of Coffeaeeae were defined as the ingroup. A
selection of genera from subfamily Isoroideae sensu stricto (i.e., excluding
‘basal Isoroideae’, after Andreasen and Bremer, 2000), were used as
outgroups. In total nine genera were used in the outgroup sample, representing the tribes Ixoroideae
and Dawson (2000), Vinckier et al. (2000), Stone and Davis (2004), Davis et al.
and Andreasen and Bremer (2000), as Ilxoroideae s.s. An overview of the genera studied in this contribution, including
their current tribal placement and general distribution, is given in Table 1.

This taxon is currently referred to (B. Sonké cf. as it superficially resembles species
of Jordan et al. (1996). For many taxa, the amplification of DNA using these
primers was not satisfactory, so we designed internal primers (Table 2) on the
basis of our first sequences in a conserved and GC-rich region suitable for
amplification of the rpl16 region in two fragments for Rubiaeaceae. The accD-
pal IGS region was amplified using the primer ACCD-769 forward and
PSA1-75 reverse from Mendenhall (1994). Two internal primers (Table 2) were
again designed to obtain satisfactory PCR products for our recalcitrant
taxa. The PCR program for the trnL-F region consisted of 2 min at 94°C followed
by 28 cycles of 1 min at 94°C (denaturation), 1 min at 50°C (annealing), and 1
min at 72°C (extension); then a final 7-min extension (72°C). For the rpl16
region, we used the PCR program: 2 min at 94°C followed by 30 cycles of 1
min at 94°C, 1 min at 52°C, and 3 min at 72°C; with a final extension at 72°C
for 7 min. The accD-psal region was amplified using the PCR program: a
denaturation phase of 3 min (94°C), followed by 30 cycles of 1 min at 94°C, 1
min at 51°C and 1 min at 72°C; and a final extension of 72°C for 5 min.
A PCR mastermix containing 2.5 mM MgCl2 (Advanced Biotechnologies,
Epsom, Surrey, UK) was used for trnL-F and rpl16 amplifications. For accD-
pal, the commercial mastermix did not give good amplifications, so we
prepared our own premix using Biotaq DNA polymerase (Bioline, UK), 10×
NH4 reaction buffer (Bioline, UK), 50 mM MgCl2, and dNTPs (Promega,
Southampton, UK). Amplified products were purified using QIAquick
purification columns (Qiagen) as described in the manufacturer’s protocol.
Cycle sequencing reactions were carried out using BigDye Terminator Mix
(Applied Biosystems, Warrington, Cheshire, UK). The program consisted of 26
cycles of 10 s denaturation (96°C), 5 s annealing (50°C), and 4 min elongation
(60°C). PCR and sequencing reactions were run using a Perkin-Elmer
(Warrington, Cheshire, UK) GenAmp model 9600 or 9700 PCR system, and
products were run on either an ABI 3100 Genetic Analyzer or an ABI
377 automated sequencer according to the manufacturer’s protocols
(Applied Biosystems). Electropherograms were edited and assembled into
tigs using Sequencer version 3.2.2 (Gene Codes Corp., Ann Arbor,
Michigan, USA). The sequences generated were submitted to GenBank using
gov/Sequin/).

**Table 2. Amplification primers for loci.**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer Forward</th>
<th>Primer Reverse</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>trnL intron</td>
<td>Forward (c)</td>
<td>5’-CGAAATCCGCTAGAGCGCTAGC-3‘</td>
<td>Taberlet et al. (1991)</td>
</tr>
<tr>
<td></td>
<td>Reverse (d)</td>
<td>5’-GGGGTAGAAGGGCATTGAACG-3‘</td>
<td>Taberlet et al. (1991)</td>
</tr>
<tr>
<td>trnL-F IGS</td>
<td>Forward (e)</td>
<td>5’-GGTCTCAGCTCCTCTATACCC-3‘</td>
<td>Jordan et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>Reverse (F1)</td>
<td>5’-GCTAGTCTAGTGTGAGTCGTTG-3‘</td>
<td>Jordan et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>Reverse (R1661)</td>
<td>5’-CCTGACCATATTTTTCCACCECGAC-3‘</td>
<td>Designed at Kew</td>
</tr>
<tr>
<td>rpl16</td>
<td>Internal Forward</td>
<td>5’-GAATAGCACTGGAGAAACGA-3‘</td>
<td>Mendenhall (1994)</td>
</tr>
<tr>
<td></td>
<td>Internal Reverse</td>
<td>5’-AGGCATGCGTCCTCTGCGC-3‘</td>
<td>Designed at Kew</td>
</tr>
<tr>
<td>accD-psal IGS</td>
<td>Forward (ACCD 769 F)</td>
<td>5’-GGCATTTTGGGACCTATAGAAAGG-3‘</td>
<td>Created at Kew</td>
</tr>
<tr>
<td></td>
<td>Forward (PSA1 75 R)</td>
<td>5’-GCTAAAAAATCTCTTTGAGGTCG-3‘</td>
<td></td>
</tr>
</tbody>
</table>
The same nine genera that were used as outgroups for the molecular analysis were scored for the morphological matrix. The total morphological variation within Ixoroideae s.s. and its tribes cannot be covered in a sample of this size, but the objective was to polarize characters for the ingroup only. Likewise, scoring of morphological characters for genera, and particularly large genera such as *Ixora*, by using one terminal, will not encompass all the variation within a genus. However, in the case of the genera we have studied in conjunction with the characters we have used, sampling of variation within genera is sufficiently representative and more than adequate for the purposes of the study. The morphological matrix provided here for Ixoroideae s.s. is the most comprehensive so far undertaken and represents a foundation for further investigation. The general distribution of character states for genera and tribes within Ixoroideae s.s. other than those sampled by us, are given in Appendix 2. The list of characters and character states, with explanatory notes (including potentially informative characters not scored in our analysis), is given in Appendix 2. The morphological matrix is given in Appendix 3. All morphological characters were treated as unordered.

Maximum parsimony was implemented to analyze (1) *trnL-F*, (2) *rpl16*, (3) *accD-psa1*, (4) combined plastid data, (5) morphological data, and (6) combined molecular and morphological data in PAUP* (Swofford, 2002). In all analyses, gaps were treated as missing data and characters were equally weighted and unordered (Fitch, 1971). The data sets were analyzed separately to identify topological conflicts: strict consensus trees were produced from each data set and compared carefully by eye. Almost no incongruence was found in the plastid analyses as far as each analysis was resolved, and the only anomaly was the placement of *Canephora* sp. (Octotropideae) sister to all *Tricalysia* (Coffeeae) in the *rpl16* analysis (BP 69). In all other analyses *Canephora* is placed with *Polyphyaerica* sp. (also Octotropideae). On the basis of this general agreement, the three plastid analyses were combined. The morphological analysis was combined with the combined plastid analysis on the basis that there was no strongly supported incongruence between the two data sets.

An additional combined molecular–morphological analysis was conducted to include the three genera (four taxa) for which we had no DNA material (*Petitiocodon, Xantonneopsis, and Nostolachma* [2 spp.]). This analysis was performed with the sole objective of elucidating the systematic position of these genera within Ixoroideae s.s.

Tree searches were conducted using 10,000 replicates of random taxon sequence addition, retaining 10 trees at each step, with tree-bisection-reconnection (TBR) branch swapping, delayed transformation (DELTRAN) optimization, MulTrees in effect, and saving a maximum of 10 trees per replicate. Support for clades in all analyses was estimated using bootstrap analysis (Felsenstein, 1985), with 10,000 replicates of full heuristic search, simple sequence addition, TBR swapping, with MulTrees in effect and saving a maximum of 10 trees per replicate. Bootstrap percentages (BP) are described as high/well-supported (85–100%), moderate (75–84%), or low (50–74%). In addition, Bremer support values (b) (Bremer, 1988, 1994; Källersjö et al., 1992), otherwise known as decay values, were obtained using PAUP* (Swofford, 2002), in conjunction with AutoDecay 4.0.2 (Eriksson, 1999), with 100 replicates of random addition for each constraint tree.

For the morphological, combined molecular analysis, and combined molecular–morphological analyses, two representatives of Vanguerieae (*Pyrostria* spp.) were used as outgroups in the analyses. This decision was based on wider studies of Ixoroideae by Andreaeen and Bremer (1996, 2000), Persson (2000), and Robbrecht and Manen (2006). The mapping of morphological characters on the morphological and morphological–molecular trees was undertaken using MacClade (version 4.07 PPC; Maddison and Maddison, 2005). Key morphological characters were mapped onto the strict consensus tree of the morphological–molecular analysis for illustrative purposes (Fig. 3).

## RESULTS

Tree data and statistics for individual analyses using (1) *trnL-F*, (2) *rpl16*, (3) *accD-psa1*, (4) combined molecular data, (5) morphological data, and (6) combined molecular and morphological data are given in Table 3. Individual plastid sequence analyses were topologically consistent and, for the purpose of the Results, Discussion, and Conclusions, are here combined and treated as a single analysis. We retrieved a significantly smaller number of trees for the analysis of *rpl16* (Table 3), although we were unable to find the reason for this anomaly. The percentage of parsimony informative characters and the values for the consistency index (CI) and retention index (RI) were similar for all plastid data sets.

### Combined analysis of plastid data

The analysis of combined plastid data resulted in four most parsimonious trees. A single most parsimonious tree with bootstrap and decay values and branch lengths is shown in Fig. 1. The genera *Argocoffeopsis, Belonophora, Calycosphochia, Calycosphochia* cf. *Coffeea, Diplospora, Discospermum, Psilanthus, Tricalysia, Sericantane, and Xantonnea* are placed within the ingroup (BP 87, b = 4). *Bertiera* is consistently placed as sister to all the other genera of the ingroup, but this relationship is very weakly supported (BP 40, b = 1). Three suprageneric clades receive bootstrap support within the ingroup: *Coffeea* and *Psilanthus* (BP 100, b = 9), *Calycosphochia, Argocoffeopsis*, and *Calycosphochia* cf. *Coffeea, Discospermum* and *Psilanthus* (BP 67, b = 1). Genus-level monophyly is supported for all genera with multiple species samples except *Coffeea, Tricalysia* (BP 98, b = 7), *Belonophora* (BP 100, b = 11), *Sericantane* (BP 100, b = 8), *Calycosphochia* (BP = 58, b = 1), *Argocoffeopsis* (BP 87, b = 2), and *Psilanthus* (BP 99, b = 4). *Coffeea* is paraphyletic with respect to *Psilanthus* (BP 86, b = 1).

### Morphological analysis

Analysis of the morphological data set resulted in 2745 most parsimonious trees; the consensus tree is poorly resolved, with few well-supported clades. The morphological analysis exhibits some topological conflict with respect to the combined molecular analysis, but...
this is limited and not well supported. The strict consensus tree with bootstrap and decay values is shown in Fig. 2. Moderate to well-supported clades include *Coffea* and *Psilanthus* (BP 79, b = 3), *Psilanthus* (BP 94, b = 3), *Belonophora* (BP 96, b = 3), *Bertiera* (BP 90, b = 3), and *Sericanthe* (BP 88, b = 2).

**Combined molecular and morphological analysis, including taxa with sequence data (42 taxa)**—Analysis of the combined molecular and morphological data set resulted in 31 most parsimonious trees. The strict consensus tree of molecular and morphological data is consistent with that of the combined molecular data tree but with less resolution at the internal nodes. Generally support values are higher. The ingroup clade receives increased support (BP 97, b = 6), for example. *Bertiera* is not the sister group of *Coffeeae*, as in the combined molecular analysis, but is instead placed with the two representatives of *Gardenieae* (BP 37, b = 1). The ingroup is divided into two main clades: *Coffea* and *Psilanthus* (BP 100, b = 13), and all other ingroup genera (BP 59, b = 1). The only other suprageneric clade receiving strong bootstrap support is that of *Calycosiphonia*, *Argocoffeopsis*, and *Calycosiphonia* cf. (BP 99, b = 9). For all genera with multiple species samples, except *Coffea*, monophyly is supported, including *Tricalysia* (BP 100, b = 10), *Belonophora* (BP 100, b = 15), *Sericanthe* (BP 100, b = 8), *Calycosiphonia* (BP 85, b = 3), *Argocoffeopsis* (BP 81, b = 2), *Psilanthus* (BP 100, b = 8), and *Bertiera* (BP 100, b = 17). *Coffea* is paraphyletic with respect to *Psilanthus* (BP 87, b = 2).

**Combined molecular and morphological analysis, including taxa without sequence data (46 taxa)**—The objective of this analysis was to deduce the systematic position for the four taxa lacking sequence data (*Nostolachma densiflora*, *N. khasiana*, *Petitioicodon parviflora*, and *Xantonnea robinsonii*). The analysis resulted in 234 most parsimonious trees. The strict consensus tree of 46 taxa is consistent with the combined molecular–morphological data tree based on 42 taxa (Fig. 3) but with some slight changes in topology, slightly lower bootstrap values, and considerably reduced decay values (Appendix S1, see Supplemental Data with online version of this article). The two species of *Nostolachma* are placed with the Asian representatives of *Coffea*, sister to *Xantonnea*; *Petitioicodon* is placed with members of *Gardenieae*, and *Xantonnea* is placed with those of *Octotropideae*, although these relationships are poorly supported.

**Distribution of morphological characters for and within the ingroup**—In the combined morphological–molecular analysis, the ingroup is supported by two morphological synapomorphies, the presence of calyculi, and a distinctly lobed style (characters 6, 23, respectively; see Appendices 2 and 3). *Bertiera* does not possess these characters. Within the ingroup, other suprageneric groupings are supported by morphological data. *Coffea* and *Psilanthus* are supported by the apparent loss of secondary pollen presentation, the presence of a hard (horny/crustaceous) endocarp, seeds with a deep ventral groove, and a seed coat consisting of crushed endotestal cells and more or less isolated fibers (characters 9, 26, 28, 29). *Calycosiphonia*, *Argocoffeopsis*, and *Calycosiphonia* cf. by the absence of a seed coat (character 29), and *Diplospora*, *Discospermum*, and *Xantonnea* (and *Nostolachma*) by the universal presence of 4-merous flowers (character 8). The monophyly of several genera is supported by morphology: *Belonophora* by the presence of imbricate calyx lobes, included anthers, and superior radicle (characters 10, 14, 21); *Sericanthe* 7–9-merous flowers, distinctly basifixed anthers, and horizontal microyle (radicle) orientation (characters 8, 15, 21); *Calycosiphonia* by having anthers with thecae (character 16); *Tricalysia* seeds with a hilar groove or shallow excavation (character 28); and *Psilanthus* by included anthers, supramedixed anthers, and included style (characters 14, 15, 22). The monophyly of *Bertiera* is supported by a bimorphic corolla tube, a distinctly peltate placenta, and an included style with longitudinal ridges (characters 12, 19, 22, 24). Key characters are mapped on Fig. 3.

**DISCUSSION**

**Close relatives of *Coffea* and their inclusion within *Coffeeae***—On the basis of plastid sequence data and morphology, the genera *Argocoffeopsis*, *Belonophora*, *Calycosiphonia*, *Calycosiphonia* cf. *Coffea*, *Diplospora*, *Discospermum*, *Psilanthus*, *Tricalysia*, *Sericanthe*, and *Xantonnea* form a well-supported clade (Fig. 3; BP 97, b = 6). With these genera included, *Coffea* is readily distinguished by the presence of calyculi and a distinctly 2-lobed style (characters 6 and 23). None of these characters are unique synapomorphies for the ingroup, although the presence of calyculi is otherwise so far only known in *Dorircera* and perhaps some species of *Ixora*. The presence of a distinctly 2-lobed style (character 23) seems to be restricted to rather few genera within *Ixoroideae* s.s. (e.g., *Ixora*, *Robbrechta De Block*). Paired axillary inflorescences (character 4) are found within several *Ixoroideae* but serve to provide a further distinguishing character for *Coffea*; paired axillary inflorescences are consistently present in *Coffea*.

*Bertiera* is sister to the ingroup in the combined plastid sequence analysis (BP 40, b = 1) but sister to two representatives of *Gardenieae* in the combined morphological–molecular analysis (BP 37, b = 1). *Bertiera* is morphologically distinct from members of *Coffea*, differing in the following characters (characters for *Coffea* in parentheses): (1) terminal inflorescences (axillary), although in three species of *Bertiera* the inflorescence is distinctly (paired) axillary (Rombrecht et al., 1994); (2) calyculi absent (present); (3) bimorphic corolla (corolla straight); (4) numerous (>30) ovules per locule (usually 1–several, but up to 20 in some species of *Tricalysia*); (5) peltate placenta (various shapes but never peltate); (6) multidirectional microyle orientation (usually downward but always in one direction); (7) distinctly ridged style (style entire) with (8) adnate lobes (lobes free). The “axillary and then terminal” position of inflorescences in some members of *Coffea* is not homologous with the truly terminal
Fig. 2. Strict consensus tree of 2745 most parsimonious trees from the morphological data set. Bootstrap values >50% are placed above the branches; Bremer support values (decay values) are given after bootstrap values. See Appendix 1 for species authorities and provenance.
inflorae of most *Bertiera* species (Davis et al., 2005). The distinctly peltate placenta in *Bertiera* seems to be unique within Ixoroideae s.s., and the genus possesses a combination of characters that is unique within this part of the subfamily, e.g. peltate placenta, numerous ovules per locule, and distinctly ridged style. In addition, most *Bertiera* species seem to have a type of exostelial thickening not found in other Rubiaceae (Robbrecht et al., 1994), although further critical study is required. *Bertiera* is the only genus of Ixoroideae s.s. that includes herbaceous species. Our findings support the removal of *Bertiera* from Coffeeae and placement in its own tribe, Bertiereae, in agreement with Bridson and Verdcourt (2003). A description and circumscription of *Bertiera* and Bertiereae is given by Bridson and Verdcourt (2003, p. 386). The placement of *Bertiera* within Coffeeae as subtribe Coffeinae as proposed by Robbrecht and Manen (2006) conflicts with the results of our analyses for both molecular and morphological data. Morphological data separate *Bertiera* from Coffeeae, whereas there are no morphological characters supporting the grouping of *Bertiera* and Coffeeae. Robbrecht and Manen (2006) provide no morphological characterization or rationale for Coffeeae including *Bertiera*. We believe that our new circumscription of Coffeeae, with the addition of new genera and the removal of *Bertiera*, is both scientifically coherent and practical.

Three generic candidates for inclusion in Coffeeae (see Table 1), viz. *Nostolachma*, *Petitiocodon*, and *Xantonneopsis* (all currently Gardenieae subtribe Diplosporinae) were not sampled for the plastid sequence analysis, and morphology alone was used to determine their systematic placement: observation of morphological characteristics and position within a combined molecular–morphological analysis (Appendix S1, see Supplemental Data with online version of this article). *Nostolachma* is placed with the Asian representatives of Coffeeae (*Diplospora*, *Discospermum*, and *Xantonnea*), which are characterized within the tribe in having consistently 4-merous flowers. This placement is not well supported, although *Nostolachma* is convincingly placed within Coffeeae. Our observations of floral morphology indicate that *Nostolachma* is dioecious, which is common in *Diplospora* and *Discospermum* but absent in the African genera of the ingroup, although some species of Madagascan *Tricalysia* are dioecious (Ali and Robbrecht, 1991; P. De Block, National Botanic Garden of Belgium, personal communication). Further study of Coffeeae genera occurring in Asia is warranted.

*Xantonneopsis* consists of a single species, *X. robinsonii* Pit. (Pitard, 1923). The combined molecular–morphological analysis (Appendix S1, see Supplemental Data with online version of this article) places *X. robinsonii* with Octotropideae (BP 69, b = 1), which is consistent with basic morphological observation: paired pedunculate, supra-axillary inflorescences and the presence of two distinctly pendulous ovules per locule arranged on a biseriate placenta. The biseriate placenta appears to be unique to Octotropideae (Robbrecht, 1988b; Bridson and Verdcourt, 2003) although it may not occur in all species within the tribe, and some genera (e.g., *Polysphaeria* Hook. f.) have a single ovule per locule. The seed coat of Octotropideae has a very distinct fingerprint-like or sometimes reticulate pattern (Robbrecht, 1988b; Bridson and Verdcourt, 2003; Stone and Davis, 2004) but unfortunately seeds of *X. robinsonii* were not available for study.

*Petitiocodon* also comprises a single species, *P. parviflorum* (Keay) Robbr.; the morphology of this species is described in detail by Robbrecht (1988b). This species does not have the key characters of Coffeeae but instead shares greater morphological affinity with members of Gardenieae. It has paired pedunculate, supra-axillary inflorescences, and a rather large broad-tubed corolla, and each locule of the bilocular ovary has a placenta attached at its base to the dividing wall of the locule with two more or less immersed ovules. The size and shape of the corolla, the position of the placental attachment, and the position of the ovules within the placenta infer a systematic position close to or within Gardenieae, although further data is required to clearly resolve the tribal placement of *Petitiocodon* within Rubiaceae. The combined molecular–morphological analysis (Appendix S1, Supplemental Data with online version of this article) supports our morphological observations, although the support values for placement within Gardenieae are very low (BP < 50, b = 1).

**Characterization of Coffeeae**—The morphological characters supporting an enlarged circumscription of Coffeeae given here are consistent with the characterization of the tribe given by Bridson and Verdcourt (2003, p. 387). One main point of difference is the nature of the apparently terminal inflorescences in Coffeeae, which has been elucidated by Davis et al. (2005). The position of the inflorescence on a short lateral shoot present in *Coffeea* subgen. *Baracoffea* (J.-F.Leroy) J.-F.Leroy, most species of *Psilanthus* subgen. *Afrocoffeea* (Moens) Bridson, and most species of *Argocoffeopsis* differs from a true terminal inflorescence. Each inflorescence originates as one of the paired axillary inflorescence and then becomes terminal on a short “shoot” or “spur” following renewed meristem activity (Davis et al., 2005, p. 410). Thus the inflorescences in Coffeeae are never strictly terminal.

The genus *Doricera*, a member of Ixoreae, is sometimes affiliated with Coffeeae (e.g., Leroy, 1989), as it has paired axillary inflorescences, calyculi, and *Coffeea*-like flowers. However, the style is club-shaped, mostly 3-lobed (not simple, and distinctly 2-lobed), the ovary is 3-locular (not 2-locular), and the seeds have a small circular excavation (“hilar cavity”) on the ventral surface (not entire or with a longitudinal invagination). Our molecular and combined molecular–morphological analyses indicate the position of *Doricera* within Ixoreae (Fig. 1: BP 100, b = 16; Fig. 3: BP 100, b = 17).

We propose an emended characterization of Coffeeae, as given next, with key characters identified with an asterisk *.


1) Trees, shrubs, woody climbers, and woody monocauls; (2) stipule pairs adnate at the base and sometimes semi-sheathing, apex entire or apiculate; (3)* inflorescences paired, axillary or axillary and then appearing terminal (by continued meristem activity of the inflorescence) on short shoots (typically in inflorescences from the previous year); (4)* inflorescences sessile (lacking a peduncle) with lowestest
calyculus in the leaf axil; (5)* calyculi (cupule-like structures formed by the contraction of shoot tissue and the reduction and fusion of leaves and stipules) present, usually 4-lobed, but sometimes 2-lobed or lobes lacking; (6) flowers 4–8–(12)-merous; (7)* corolla tube narrow and straight and (8) with lobes overlapping to the left (Coffeea-like flowers); (9) anthers exerted, rarely included; (10) stigmatic lobes usually excluded but sometimes distinctly included (Psilanthus), and included or excluded in dioecious species; (11) ovary 2-locular, placentation axile; (12) each locule with the placenta attached to the septum, at the middle or just above, rarely near the apex (only Belonophora and Sericantes); (13)* ovules usually 1 or 2 per locule or up to 10 (rarely c. 20); (14) ovules on a small placenta, or placenta large and ovules either on or semi-embedded within the placental tissue; (15) embryo radicle usually inferior, rarely horizontal (only Sericantes), or superior (only Belonophora); (16) style simple (lacking specialized features), glabrous, *distinctly 2-lobed; (17) fruit an indehiscent drupe, with few (1 or 2) to several seeds (rarely up to c. 10); (18) ventral surface of seed more or less entire, (sometimes with a shallow hilar groove or shallow excavation), or with a distinct longitudinal ventral invagination ("coffee-bean" morphology: only Coffeea and Psilanthus); (19) endosperm usually entire but sometimes ruminate (only some species of Tricalysia and Coffeea); (20) testa cells isodiametric or elongated, thickenings present or absent, smooth along outer tangential walls, or (in Coffeea and Psilanthus) with cells crushed during the development of endosperm and composed of thin elongate parenchymatic cells usually containing many more or less isolated fibers; (21) pollen (2–)3–5-colporate (zonocolporate).

Genera included Argocoffeopsis, Belonophora, Calycosphonia, Coffea, Diplospora, Discospermum, Nostolachma, Psilanthus, Sericantes, Tricalysia, and Xantonnea. The entity Calycosphonia cf. may represent an additional genus for the tribe; further material and morphological investigations are required.

Further details of salient characters are given in Appendix 2. In addition to the findings presented here, this morphological characterization is based on data derived from Robbrecht (1981), Robbrecht and Huff (1986), Bridson and Verdcourt (2003), Davis et al. (2005), and for pollen, Stoffelen et al. (1997).

**Relationships within Coffeeae**—Based on combined molecular data (Fig. 1: BP 100, b = 9) and combined molecular–morphological data (Fig. 3: BP 100, b = 13), there is strong support for a relationship between Coffeea and Psilanthus. This is to be expected, given that they share a carpel morphology that is unique within Ixoroideae s.s. (see Fig. 3; Introduction; Appendices 2 and 3). They are also peculiar within Ixoroideae s.s. in lacking secondary (ixoroid) pollen presentation, which is present in nearly all members of the subfamily (Puff et al., 1996), although the distribution of this character in some monoeocious members of Ixoroideae (e.g., Diplospora and Discospermum) is still unclear (e.g., Ali and Robbrecht, 1991).

A close phylogenetic relationship between *Coffeea* and *Psilanthus* based on molecular and morphological data is concomitant with close cytological correspondence (Lombello and Pinto-Maglio, 2003, 2004), including the production of a fertile intergeneric hybrid (Couturon et al., 1998). Our results are also in agreement with the phylogenetic studies of Lashneres et al. (1997), Cros et al. (1998), Andreasen et al. (1999), and Andreasen and Bremer (2000, fig. 3), who showed that *Psilanthus* is nested within *Coffeea*. Detailed species-level studies are now required to assess the phylogenetic relationship between *Coffeea* and *Psilanthus*.

The combined molecular–morphological analysis shows that there is strong support (Fig. 3: BP 99, b = 9) for a clade containing Argocoffeopsis, Calycosphonia, and Calycosphonia cf. distinguished by the lack of a testa. Earlier studies did not identify close phylogenetic links between Argocoffeopsis and Calycosphonia (Robbrecht, 1981; Robbrecht and Huff, 1996), although it has been known for some time that these genera lack a testa (e.g., Robbrecht and Huff, 1996) and that this feature is all but absent within Coffeeae and Gardenieae subtribe Diploropininae (Ali and Robbrecht, 1991) and rare in Ixoroideae s.s. (Robbrecht and Huff, 1986). Ali and Robbrecht (1991) report that in Diplospora (only D. wrayi King & Gamble) the mature exotesta cells are very rarely crushed by the development of the endosperm, so that the seed coat is reduced to a thin pellicle, as in Argocoffeopsis and Calycosphonia. The exact systematic position of Calycosphonia cf. has not been elucidated by our study. It generally looks much more like Calycosphonia than Argocoffeopsis, although it can be excluded from the former genus on the basis of lacking anther thecae and having 5-merous flowers (7- to 8-merous in *Coffeea*). Calycosphonia cf. also closely resembles one species of Argocoffeopsis, A. lembilini (A. Chev.) Robbr. Most species of Argocoffeopsis are woody climbers, but A. lembilini [and A. rupestris (Hiern) Robbr.] are shrubs or small trees. The only readily discernible difference between Calycosphonia cf. and A. lembilini is that the former has large ellipsoid fruits (seeds elliptic in outline) and the latter has small spheroid seeds (seeds circular in outline). However, further study is required.

The combined molecular–morphological analysis weakly supports a relationship between three of the four Coffeeae genera occurring in Asia: Diploropina, Discospermum, and Xantonnea (Fig. 3; BP 64, b = 1), and the fourth, Nostolachma, is placed within this group based on morphology alone (Appendix S1, see Supplemental Data with online version of this article). This group is characterized by the presence of 4-merous flowers, a character unique to these four genera within Coffeeae (discussed earlier). This is the first time a relationship between these four genera has been suggested. These results agree with Robbrecht and Huff (1986), who concluded that Nostolachma does not have a close relationship with *Coffeea* and *Psilanthus* as postulated by Leroy (1980). The recognition...
of *Diplospora* and *Discospermum* (from Asia) as separate from *Tricalysia* (Africa and Madagascar) by Ali and Robbrecht (1991) is supported by our study. According to Ali and Robbrecht (1991), Asian *Diplospora* and *Discospermum* can be separated from African and Madagascan *Tricalysia* (characters for *Tricalysia* in parentheses) by standard type colleters (mostly with modified colleter type); almost consistently 4-merous flowers (predominantly 5-pleiomerous); inconspicuous bracteoles/calyculi (large, sheathing bracteoles resembling the calyx); small flowers (larger flowers); strong tendency toward unisexual flowers (hermaphrodite; unisexual in Madagascan species); secondary pollen presentation possibly absent (generally or universally present); seed coat mostly with thickened exotestal cells (not thickened). Three of these characters are included in our morphological matrix (see Appendices 2 and 3). The other Asian genus, *Xantonnea*, was included in the tribe Octotropideae by Robbrecht (1988b, 1994), but our molecular data and the presence of key morphological features place it with the genera of Coffeeae restricted to Asia (Figs. 1, 3).

Given the low branch support values (BP = 57, b = 1) and low level of internal resolution for the clade that includes *Tricalysia*, *Belonophora*, *Sericanthe*, and the Asian Coffeeae (*Diplospora*, *Discospermum*, and *Xantonnea*), further data are needed to fully elucidate the systematic relationships for and within this clade. In addition, the relationship of the *Argocoffeopsis-Calycosiphonia-Calycosiphonia* cf. clade with all of the above genera needs further investigation.

**The monophyly and circumscription of genera of Coffeeae**—Combined molecular and morphological data (Fig. 3) are consistent with the assumed monophyly of *Tricalysia* (BP = 100, b = 10), *Belonophora* (BP = 100, b = 15), *Sericanthe* (BP = 100, b = 8), *Calycosiphonia* (BP = 85, b = 3), *Argocoffeopsis* (BP = 81, b = 2), and *Psilanthus* (BP = 100, b = 8). However, for some genera we have only sampled a relatively low number of species per genus. In particular, *Tricalysia* (98 spp.) and *Coffea* (95 spp.) require further sampling. *Coffea* is paraphyletic with respect to *Psilanthus* (see earlier). The monophyly of the Asian Coffeeae genera could not be tested in our study due to a lack of suitable DNA material. Molecular and morphological data are needed to test the monophyly and elucidate the systematic relationships of *Diplospora*, *Discospermum*, *Nostolachma*, and *Xantonnea*. It is likely that some generic synonymy may result from further studies.

Apart from the Asian genera, the monophyly of most genera of Coffeeae is supported by one or more synapomorphies (see earlier). The exceptions are *Argocoffeopsis*, which is based on a combination of characters (e.g., Bridson and Verdcourt, 2003), and *Coffea* (Davis et al., 2005).

*Coffea* differs from *Psilanthus* in its long, exerted style (short, included in *Psilanthus*) and submedified (vs. supra-medified) excluded (vs. included) anthers. According to Davis et al. (2005), there are two other morphological differences between these two genera [excluding *P. melanocarpus* (Welw. ex Hiern) J-F. Leroy]. *Coffea* has short to long anther filaments (very short to more or less absent filaments in *Psilanthus*) and pollen with 3 or 4, rarely 2, apertures (vs. 4 or 5, rarely 3). Despite these morphological distinctions, molecular, morphological, and combined analyses all show *Psilanthus* as derived within the *Coffea* clade (Figs. 1, 3), so that the recognition of *Psilanthus* results in a paraphyletic *Coffea*.

**Conclusions**—The cladistic analyses presented here provide the most inclusive systematic study of Coffeeae to date, with molecular sequence data for all but one of the component genera. The enlargement of Coffeeae by the addition of *Diplospora* and *Tricalysia*, on the basis of molecular sequence data (Andreasen and Bremer, 1996, 2000; Persson, 2000) is supported by our study. The addition of *Discospermum* and *Sericanthe* on the basis of morphological inference (Andreasen and Bremer, 2000) is also confirmed. Preliminary results from this study (A. P. Davis, unpublished data) were used to support the transfer of *Argocoffeopsis*, *Belonophora*, and *Calycosiphonia* to Coffeeae by Bridson and Verdcourt (2003), and these transfers are again confirmed by our completed study. *Xantonnea* is transferred from Octotropideae (Robbrecht, 1988b, 1994) to Coffeeae on the basis of molecular sequence data and morphology. *Nostolachma* is transferred from Gardeniaeae subtribe Diplosporinae (Robbrecht, 1988b, 1994) to Coffeeae on the basis of morphological data. *Petitiododon* and *Xantonneopsis* are not members of Coffeeae; based on morphological data, the former is placed tentatively in Gardeniaeae and the latter Octotropideae.

We propose that Coffeeae should include 11 genera, viz. *Argocoffeopsis*, *Belonophora*, *Calycosiphonia*, *Coffea*, *Diplospora*, *Discospermum*, *Nostolachma*, *Psilanthus*, *Tricalysia*, *Sericanthe*, and *Xantonnea* (see Table 1), and have given an updated morphological characterization of the tribe (see earlier). A poorly known and informal taxon, *Calycosiphonia* cf. is also placed in Coffeeae, although its generic status has yet to be confirmed (A. P. Davis and B. Sonké, personal communication).

*Coffea* and *Psilanthus* are clearly distinct within Coffeeae, and a sister relationship to the rest of the tribe is inferred but is only weakly supported by our combined molecular–morphological analysis (Fig. 3: BP = 59, b = 1). Within other Coffeeae, a well-supported phylogenetic relationship is inferred for the species lacking a discernible seed coat (*Argocoffeopsis*, *Calycosiphonia*, and *Calycosiphonia* cf.). The monophyly of *Argocoffeopsis*, *Belonophora*, *Calycosiphonia*, *Psilanthus*, *Tricalysia*, and *Sericanthe* is consistent with our analyses; one or more clear-cut synapomorphies are identified for *Belonophora*, *Calycosiphonia*, *Psilanthus*, and *Sericanthe*. *Coffea* is paraphyletic, as identified in other molecular analyses (Lashermes et al., 1997; Cros et al., 1998; Andreasen et al., 1999; Andreasen and Bremer, 2000, fig. 3). Morphological separation of *Coffea* and *Psilanthus* is possible (Davis et al., 2005; and see Discussion), but further data, including wider sampling in these genera, are now urgently required to ascertain whether two genera can be upheld.

The exclusion of *Bertiera* from Coffeeae and its placement in its own tribe (Bridson and Verdcourt, 2003, p. 386), *Bertiereae*, are supported by our combined molecular (Fig. 1), combined molecular–morphological data (Fig. 3), and our morphological survey. The inclusion of *Bertiera* within Coffeeae, as subtribe Bertierinae as proposed by Robbrecht and Manen (2006) is not supported by our data. Coffeeae becomes a strictly Old World tribe following the exclusion of *Bertiera*. The decision to place Gardeniaeae subtribe Diplosporinae into the synonymy of *Coffea* (Bridson and Verdcourt, 2003) is substantiated by our analyses.

**LITERATURE CITED**

March 2007] DAVIS ET AL.—CIRCUMSCRIPTION AND PHYL OGENY OF COFFEEAE 325


Mendennalli, M. 1994. Phylogeny of Baptisia and Thermopsis (Legumi- nosae) as inferred from chloroplast DNA and nuclear ribosomal DNA sequences, secondary chemistry, and morphology. Ph.D. dissertation, University of Texas, Austin, Texas, USA.


Persson, C. 2000. Phylogeny of Gardenieae (Rubiaceae) based on
chloroplast DNA sequences from the rps16 intron and trn(L(UAA)-F(GAA)), \textit{Nordic Journal of Botany} 20: 257–269.


APPENDIX 1. Voucher information and GenBank accession numbers for taxa used in this study. A dash indicates the region was not sampled. Voucher specimens are deposited in the following herbaria: K = Royal Botanic Gardens, Kew; TAN = Parc de Tsimbazaza, Antananarivo; BR = National Botanic Garden of Belgium, Meise; YA = National Herbarium of Cameroon, Yaoundé.
1. **Habit:** 0 = tree or shrub; 1 = climber (liana, vine); 2 = herb. *Bertiera* (Bertiereae) is the only member of Ixoroideae s.s. to have herbaceous representatives. Climbing habit is also present in Bertiereae, Cremasporeae, Gardenieae, Pavetteae, and Vanguerieae.

2. **Petioles:** 0 = not articulated; 1 = articulated. *Petioles* commonly articulated in Ixoreae and Octotropidaceae, although this character is not consistent within these tribes and may also occur randomly in Ixoroideae and other Rubiaceae genera.

3. **Bacterial nodules:** 0 = absent; 1 = present. *Bacteria* nodules are almost universally present in Sericanthaceae and Pavettaceae (Pavetteae); *Pavetta* was not included in the matrix.

4. **Inflorescence position:** 0 = axillary or axillary and then terminal via continued meristematic activity of the inflorescence, paired at nodes; 1 = terminal or terminal-overtopped (“pseudo-axillary”). *Fixed within many tribes: always axillary in Coffeae, Cremasporeae, Octotropidaceae, Vanguerieae; terminal in Alberteae (not included in matrix), Bertiereae (three species axillary), Ixoreae, and Pavetteae; variable within Gardenieae. Some of this variation is included within the matrix.**

5. **Inflorescence:** 0 = sessile (lacking a peduncle); 1 = pedunculate. *Variable within all tribes, except Coffeae and Cremasporeae.*

6. **Calyciul:** 0 = present; 1 = absent. *Restricted to Coffeae and Doricera (Ixoreae), and one or two species of Asian *Ixora* (Ixoreae).*

7. **Flowers:** 0 = monoeccious (hermaphrodite); 1 = dioecious (single sexed). *Variable within many tribes (Coffeae, Gardenieae, Ixoreae, Octotropidaceae, Vanguerieae).*

8. **Flowers (merosity):** 0 = 4-merous (exclusively or predominantly); 1 = 5–6-merous; 2 = 7–9-merous (rarely 12-merous). *Very variable within tribes but fixed, or more or less so, in some genera (e.g., Diplospora, Discoespernum, Nostolachma, Xantonnea (Coffeae), *Ixora* (Ixoreae), which are predominantly or exclusively (respectively) 4-merous.*

9. **Secondary (Ixorid) pollen presentation (SPP):** 0 = absent; 1 = present. *Variable within Coffeae and Gardenieae and possibly other tribes, but generally dominant in Ixoroideae s.s. Robberecht and Puff (1986) reported that SPP is present in *Coffea* and *Psilanthus,* but this was contested by Bridson and Verdcourt (1998, 2003), Puff et al. (1996), and Andreassen and Bremer (2000). Robberecht and Puff (1986) report SPP lacking in *Belonophora;* this was challenged by Cheek and Dawson (2000).

10. **Calyx lobes:** 0 = absent or free; 1 = imbricate. *Probably restricted to very few genera of Ixoroideae s.s., although no thorough survey has been undertaken. A similar calyx lobe arrangement occurs in some species of *Tricalysia* (for summary, see Bridson and Verdcourt, 1988, 2003), but this was not scored in the morphological matrix (Appendix 3).*

11. **Corolla aestivation:** 0 = overlapping (contorted) to left; 1 = overlapping (contorted) to right; 2 = not overlapping (valvate). *Left-contorted corollas are found within nearly all Ixoroideae s.s., apart from Gardenieae, where right-contorted corollas are present in three genera (i.e., Hyperacanthus, Philocalyx Bridson, and some species of Rothmannia Thunb.) and Gardeniopsis Miq. (tribal placement unknown). Vanguerieae have valvate aestivation.*

12. **Corolla shape:** 0 = More or less straight, or widening toward apex; 1 = tube bimorphic (circum-constricted: usually above the first half of the tube). *Fixed within most tribes but variable within Gardenieae and perhaps some Vanguerieae.*

13. **Corolla (hairs in throat):** 0 = absent; 1 = present. *Variable within all tribes and many genera but always absent in some genera and generic groupings (e.g., *Coffea* and *Psilanthus*). Further study is required.

14. **Anthers:** 0 = emergent; 1 = partially emergent; 2 = included. *Variable within most tribes of Ixoroideae s.s. and some genera (e.g., *Argocoffeopsis*).*

15. **Anthers (number of ovules per locule):** 0 = 0; 1 = 1–20; 2 = 21–30; 0 more. *Variable within tribes and genera but few ovules (1–10) and many ovules (≥30) rarely found within genera and closely related genera, except some Gardenieae (Robberecht and Puff, 1986; Rakotonasolo and Davis, 2006), and Octotropidaceae (Robberecht and Puff, 1986; Stone and Davis, 2004).*

16. **Gynoecium (number of ovules per locule):** 0 = 0; 1 = 1–20; 2 = 21–100; 0 more. *Variable within tribes and genera but few ovules (1–10) and many ovules (≥30) rarely found within genera and closely related genera, except some Gardenieae (Robberecht and Puff, 1986; Rakotonasolo and Davis, 2006), and Octotropidaceae (Robberecht and Puff, 1986; Stone and Davis, 2004).*

17. **Gynoecium (placental attachment):** 0 = middle, or slightly below; 1 = apical or near apex (or from apex, downward if more than one ovule; placenta[s] always pendulous); 2 = basal; 3 = along length of placenta or more or less so. *Variable within tribes of Ixoroideae s.s., or fixed (Vanguerieae, Cremasporeae); usually fixed within genera.*

18. **Gynoecium (placentation):** 0 = small or inconspicuous; 1 = hemi-circular to ± hemi-ellipsoidal; 2 = distinctly peltate; 3 = linear; 4 = a soft pulpy matrix. *Variable within Gardenieae, although a pulpy matrix is predominant in this tribe. In Octotropidaceae the placenta is nearly always linear and biseriate, if there is more than one ovule per locule. Hemi-circular to more or less hemi-ellipsoid placetas predominant in most other Ixoroideae s.s.; peltate placentas are restricted to Bertiera (Bertiereae), although study of poorly known genera is required.*

19. **Gynoecium (placenta):** 0 = 0; 1 = 1–10; 2 = 11–30; 0 more. *Variable within tribes of Ixoroideae s.s., or fixed (Vanguerieae, Cremasporeae); usually fixed within genera.*

20. **Gynoecium (micropyle orientation):** 0 = downward (radicle inferior); 1 = horizontal; 2 = upward (radicle superior); 3 = variable, in many directions (numerous ovules). *Character states 0, 1 and 2 variable within Coffeae and Pavetteae; and somewhat
variable within Octotropideae (A. Davis, personal observation); fixed in Cremasporeae (inferior), Ixoreae, Vanguerieae (Appendix 3); usually fixed within genera of Ixoroideae s.s. Taxa with multiple ovules per locule (Bertiera (Bertiereae) and most Gardeniaeae) have variable micropyle orientation.

22. **Style:** 0 = emergent (exserted); 1 = included. * Variable within tribes, although almost always emergent to some degree in Ixoreae, Cremasporeae, Bertiereae, Octotropideae, Pavetteae, and Vangerieae. Notably variable in Gardeniaeae and to a lesser extent Coffeeae.

23. **Style:** 0 = distinctly 2-lobed; 1 = lobes adnate for their entire length, or free at apex (2-, 3- or many-lobed). * Fixed within most tribes but variable within Gardeniaeae and Pavetteae (e.g., see Bridson, 1988) and Ixoreae.

24. **Style:** 0 = simple; 1 = with distinct longitudinal ridges. * Consistent within Bertiereae (Bertiereae) (Robbrecht et al., 1994), also found in some genera of Octotropideae (Bridson, 1988; A. Davis, personal observation for Madagascan taxa), Gardeniaeae and Pavetteae (e.g., see Bridson, 1988), but probably absent in all other Ixoroideae s.s. Variation within the outgroup was not recorded in the morphological matrix (Appendix 3).

25. **Style:** 0 = glabrous; 1 = hairy. * Variable within Ixoroideae s.s., present but not universal in Cremasporeae, Gardeniaeae, Ixoreae, and Pavetteae. Common or perhaps universal in Octotropideae; always glabrous in Coffeeae, and perhaps Albertiaeae. Possibly universally glabrous in Vangerieae. Further critical study is required.

26. **Pyrene:** 0 = endocarp weakly developed, thin or soft; 1 = endocarp hard (horny/crustaceous); 2 = endocarp woody. Character state 1 appears to be restricted to Coffea and Psilanthus; all other genera of Ixoroideae s.s. with endocarp weakly developed, thin or soft, apart from Vangerieae, which is woody and tough.

27. **Pyrene:** 0 = lacking preformed germination slits; 1 = apical preformed germination slits present. Present in Vanguerieae; absent in all other Ixoroideae s.s. apart from some members of Albertiaeae.

28. **Seeds: (ventral [adaxial] surface):** 0 = entire or more or less so; 1 = with a hiliar groove or shallow excavation; 2 = with a distinct hila cavity; 3 = with a deep ventral groove (i.e., *coffee-bean morphology*); 4 = with a notch, or distinct lateral groove (or similar) at or above the midpoint. * More or less consistent within tribes, except Coffeeae: Coffea and Psilanthus have a deep ventral groove, Tricalysia has either a shallow hiliar groove or shallow excavation or an entire ventral face, and other Coffeeae are entire. Vanguerieae seed morphology is consistent and highly characteristic.

29. **Seed coat:** 0 = consisting of crushend endotestal cells and more or less isolated fibers (sclereids); 1 = consisting of exotesta cells, without fibers (sclereids); 2 = absent. * State 0 apparently restricted to Coffea and Psilanthus; state 1 found in most Ixoroideae s.s. Within Ixoroideae s.s., state 2 is restricted to Argocoffeopsis, Calycosiphonia, Calycosiphonia cf., and one species of Diplospora (only D. wrayi; Ali and Robbrecht, 1991). Further study is required.

30. **Seed coat:** 0 = testa appearing ± smooth at low magnification (×10); 1 = testa appearing fibrous with an obvious fingerprint-like pattern at low magnification (×10). * State 1 restricted to Octotropideae. Preliminary study indicates that this feature may be universal in Octotropideae after recircumscription of the tribe (A. Davis, personal observation).

31. **Pollen:** 0 = colporate; 1 = porate. * All Ixoroideae s.s. have colporate pollen grains, except Gardeniaeae (which may be colporate, colpodporate, or porate). Most Ixoroideae have 3-colporate pollen, but see following notes for Coffea and Psilanthus.

Notes: Other characters of potential systematic importance were reviewed but omitted from the data matrix for various reasons. The number of pollen apertures (Lobreau-Callen and Leroy, 1980; Chinnappa and Warner, 1981; Stoffelen et al., 1997) may have systematic utility, but this character was not scored in our morphological matrix due to high levels of polymorphism, overlap between Coffea and Psilanthus, and missing data. The nature of thickening in exotestal walls in Gardeniaeae and related tribes has been investigated in detail by Robbrecht and Puff (1986). We could not devise a satisfactory method for scoring these characters and the character states; moreover, there seems to be considerable polymorphism within genera, and there is much missing data. Despite these concerns, we believe that further study of thickening in exotestal cell walls is warranted. The presence and type of pollen orbicules may also provide systematic data, because Vinckier et al. (2000) have shown that the same pollen orbicule type (type VI) was found in Coffea, Psilanthus, and Bertiera, but no other member of Ixoroideae. The incompleteness of their data meant that we could not include this character in the morphological matrix. Stipule characters (e.g., distinctly awned vs. slightly awned or entire, and degree of sheathing), may have systematic utility within Coffeeae and Ixoroideae, but so far a detailed investigation of stipule morphology has not been undertaken. Colletor type, i.e., standard vs. modified (Robbrecht, 1988b; Ali and Robbrecht, 1991), also requires further sampling and study.

Some characters are relatively well known as having systematic utility in Ixoroideae although they were not scored in our analysis because the restricted sample size means they would be autapomorphic for some terminals. These characters include placentation, which is axile in all members of Ixoroideae apart from the genera of Gardeniaeae that are parietal (e.g., Gardenia), and placentation also may be variable within Gardeniaeae genera (Rakotonasolo and Davis, 2006). Another example is pollen arrangement in Gardeniaeae, in which pollen can be in monads, tetrads and polyads. In our analysis tetrads would be autapomorphic for Gardenia.
## APPENDIX 3

Morphological matrix of 31 selected characters for 46 operational taxonomic units (OTUs) of potential Coffeeae and outgroups.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Character states</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coffea kapakata</strong></td>
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<td><strong>Coffea mangoroensis</strong></td>
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</tr>
<tr>
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</tr>
<tr>
<td><strong>Tricalysia sp. 3</strong></td>
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<tr>
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<td><strong>Sericanthe sp.</strong></td>
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<tr>
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<tr>
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</tr>
<tr>
<td><strong>Izora sp.</strong></td>
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